

Liquen *Usnea densirostra* como bioindicador de metales pesados. Determinación por ICP-AES acoplado con nebulizador ultrasónico

Heavy metals determination by ICP-AES coupled with ultrasonic nebulization using the lichen Usnea densirostra (Tayl.) as biomonitor pollution in San Luis, Argentina

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RESUMEN

El liquen *Usnea densirostra* (Tayl.) fue transplantado durante el periodo de Diciembre 1997 - Noviembre 1998 en 20 sitios de biomonitorio en el microcentro de la ciudad de San Luis, Argentina. La concentración de metales pesados fue determinada en los líquenes usando Espectrometría de Atomización Atómica Electrotermica (ETAAS) y Espectrometría de Emisión Atómica Acoplada Inductivamente a Plasma (ICP-AES), asociada con un sistema de nebulización ultrasónica. Los resultados indican que este liquen puede ser usado para determinar la deposición de contaminantes aéreos, en ambientes semiaridos.

Palabras Claves: Liquen. Bioacumulador. Metales Pesados. ICP-AES. Nebulización ultrasónica.

ABSTRACT

The lichen Usnea densirostra (Tayl.) was transplanted during the period December 1997 – November 1998 to 20 biomonitoring sites in the downtown of San Luis city, Argentina. Concentration of heavy metals was determined in the lichen using Electrothermal Atomization Atomic Spectrometry (ETAAS) and Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES) associated with an ultrasonic nebulization system. The results indicate that this lichen species may be used to determine deposition patterns of air pollutants in semi-arid environments.

Keywords: Lichen. Biomonitoring. Heavy metals. ICP-AES. Ultrasonic nebulization.

INTRODUCTION

The lichens consist of both an alga and a fungus. The alga contains chlorophyll and provides the lichen with nutrients by photosynthesis, and the fungus absorbs water and nutrients from surroundings. As a result, lichens are self-sufficient and can grow on rocks, roofs, tree trunks, and similar surfaces. Due to the fact that they have no roots, lichens absorb their nutrients from the air around them, instead of

doing so from the soil as plants do (Arms 1990). Consequently, when nutrients are absorbed into the thallus, other elements, not necessarily required for growth, are also absorbed. Heavy metal cations are absorbed, in part via exchange mechanisms, and the exchangeable fraction decreases when the thalli become air-dried. It has been reported that in humid regions, a high correlation exists between levels of air pollutants and

injury to the living cell and tissues of lichens (Dillman 1996).

Rope and Pearson (1977) described lichens as air pollution biomonitors in a semi-arid environment in a place similar to the one described in this paper. The city of San Luis (latitude 33° 16' S. longitude 66° 21' W) is located in the central western part of Argentina in a region called Cuyo which comprises the provinces of Mendoza, San Juan, La Rioja and San Luis.

The urban area is situated on the southwest of San Luis mountains. It shows an annual rainfall of 579.9 mm, the average annual temperature is 17.4°, the average annual humidity is 59%, and average annual wind speed is 13 km/h, with predominance of northern and southwestern winds. A quick assessment of demographical data indicates that population growth rates have accelerated in the past years. The 1991 National Census reported a population of 110,353 inhabitants, which, considering the figures of the previous Census (1980: 70,999) indicates a population growth rate of 43%, a high value for Argentina. These 40,000 new inhabitants have deepened the gap between population and services.

There remain vegetation areas of the boundary Prosopis Forest and Arid Chaco in the periphery of the urban area. These are partially degraded by timber extraction, but species such as

Prosopis flexuosa, *Jodina rombifolia*, *Larrea divaricata*, *Geoffroea decorticans*, among others, can be found (Lijteroff et al. 1999).

Although ETAAS and ICP-AES associated to conventional neumatic nebulization are the most used techniques in the determination of trace of heavy metals, the low levels of this element concentration in lichens are not compatible with the determination limits of these techniques. One way to improve the sensitivity of ICP-AES consists of associating this technique with ultrasonic nebulization (USN) (Seiler and Sigel 1994; Minoia et al. 1994). Due to the elevated efficiency of this technique compared with traditional neumatic systems, USN seems able to support the ICP-AES in many interesting fields of application, especially in the area of environmental control and for toxicology applications. Besides, the use of an ultrasonic nebulizer can provide a 5-50 fold improvement in detection limits (Minoia et al. 1994; Teissèdre et al. 1998; Pérez-Jordan et al. 1998).

In the present work, a method for determination of heavy metals in lichens using ICP-AES associated with an ultrasonic nebulization system (USN) is proposed. This heavy metals were determined in samples lichen *Usnea densirostra* (Tay.) after an adequate optimization of the system operating conditions.

MATERIALS AND METHOD

The lichen *Usnea densirostra* was collected in July 1997 from El Trapiche (situated 50 km N.E. of San Luis), known to be "unpolluted". Twigs covered with the lichen were transplanted immediately to 20 different biomonitoring stations (Figure 1) as has been described in Ferry and Coppins (1979). Simultaneously, some of these twigs were transplanted back to the control site in Balde (W. San Luis). The lichen material collected downtown during different seasons of the year were divided into 3 samples per site (each sample consisting of several thalli). The thalli were rinsed with distilled water in order to eliminate dust, and then dried, wet ashed, and prepared following this procedure: 1.5 g of thalli was weighed and put into a glass vessel and dried in store during one night to 90 ° C. Each sample was moistened with 15 mL of HNO₃ (c) in portions of 3 mL each were added. The sample was headed in a sand

bath up to dryness. At last, the residue was taken with doubly distilled water and placed into a water bath during a 12 h period. After being filtered through a S&S paper, the filtrate was made up to 25 mL with doubly distilled water. The operating conditions were established and the determination was carried out. The concentration of Ni, Cr, Cu, Zn, and Pb were performed with a sequential inductively coupled plasma spectrometer [Baird (Bedford, MA, USA) ICP 2070]. The 1 m Czerny – Turner monochromator had a holographic grating with 1800 grooves mm⁻¹. An ultrasonic nebulizer U-5000 AT (CETAC Technologies) was used. The ICP and ultrasonic nebulizer operating conditions are listed in Table 1. A schematic representation of the analytical system used is shown in Figure 2. Tygon type pump tubes (ismatec, Cole-Parmer Instrument Company, Illinois, USA) were employed to propel the sample. The heavy metals

were determined by the following spectral line:
 Cu – 327.396 nm; Zn – 213.856 nm; Cd – 228.802;
 Ni – 221.647 nm and Pb – 220.353 nm.

All chemicals reagents were of analytical grade purity and the solutions taken to volume with doubly distilled water.

FIGURE 1. Map of the microcenter of the city with the different sampling points

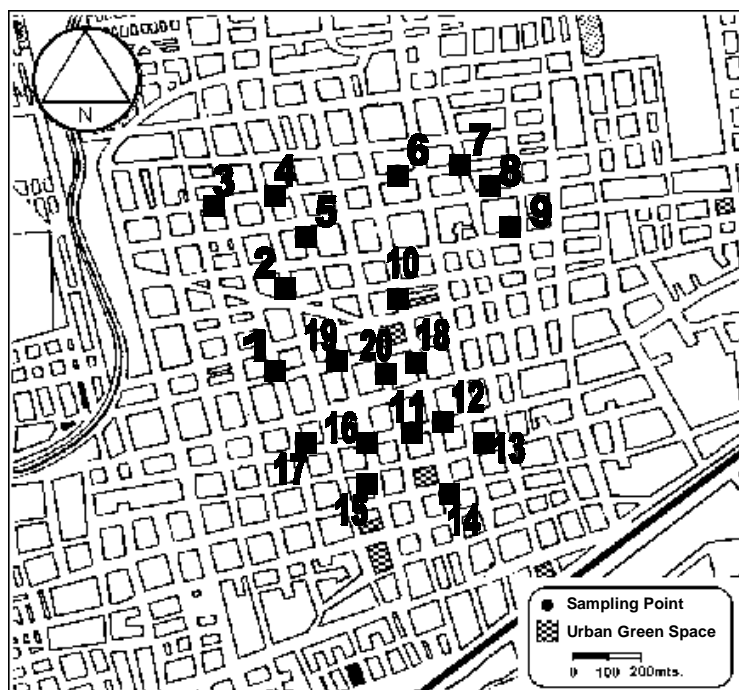
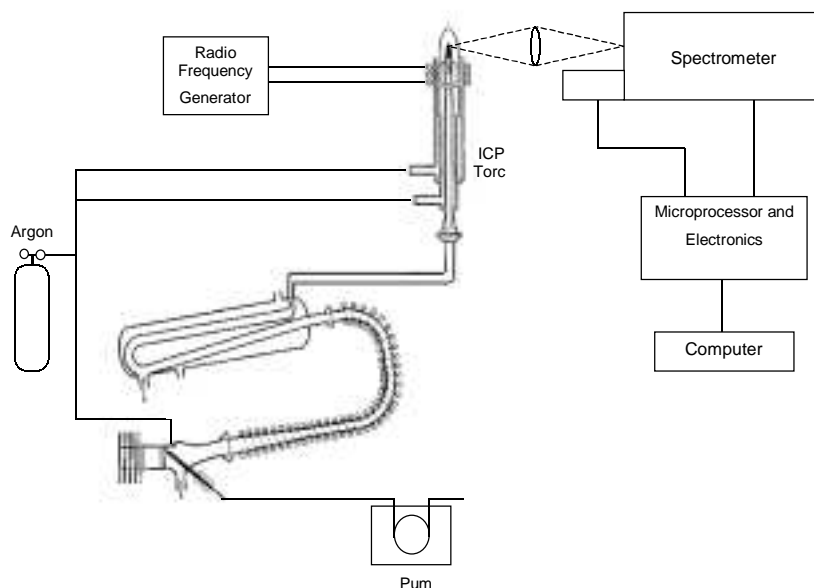


TABLE 1. ICP and ultrasonic nebulizer instrumental parameters

ICP conditions	
RF generator power	1 kW
Frequency of RF generator	40.68 MHz
Plasma gas flow rate	8.5 L min ⁻¹
Auxiliary gas flow rate	1.0 L min ⁻¹
Observation height (above load coil)	15 mm
Ultrasonic nebulizer conditions	
Heater temperature	140 °C
Condenser temperature	2 °C
Nebulizer gas flow rate	0.95 L min ⁻¹

FIGURE 2. Schematic diagram of the instrumental set-up. S: sample solution; N: ultrasonic nebuliser; W: waste; H: heater; C: condenser



RESULTS AND DISCUSSION

Comparing the average values there are not significant, statistical differences between the four seasons studied for the different metals. It could be proven that in the four stations statistically significant differences don't exist, among the transplanted lichen in the microcenter and the gathered lichen of the pristine area, for lead and cadmium.

Although some years ago the use of leaded fuel, it was habitual which was used as antiknock, at the present time this element has been substituted by others such as iron or hexanes that perform identical function and don't produce noxious effects on the living beings. Due to this change in the constituents of the fuels they have been called ecological. The latter would explain the fact that lead has been detected in

concentrations above the normal quantity at the sampling points located in San Luis' city.

Cadmium is liberated by the normal waste suffered by tyres, and it was not found in concentrations higher than normal. Data obtained for lead and cadmium concentrations are positive, as showed in Tables II and III. These facts point out that in San Luis' city the emission of these metals is extremely low and can be absorbed by the natural atmosphere without evidencing dysfunctions of any type. However new emission focuses can be generated, for example industries that liberate these elements and they affect San Luis' city, or the increasing in production. A similar situation would happen in the case of increasing the traffic.

TABLE II. Concentration of Cadmium (ppm of dry matter).

Point of Sampling (n=3)	season			
	summer	autumn	winter	spring
1	2.67	3.24	3.07	2.91
2	3.69	2.48	2.74	4.36
3	3.47	3.78	3.94	3.73
4	4.24	4.45	3.75	4.07
5	3.05	3.86	3.45	2.61
6	3.04	3.80	3.02	4.01
7	3.06	3.92	3.12	2.68
8	4.23	2.40	3.10	2.25
9	3.48	3.32	3.04	2.87
10	4.25	4.40	3.90	2.91
12	3.70	3.29	3.12	2.61
13	3.68	3.83	2.70	3.73
14	3.46	2.43	3.92	4.07
15	2.65	4.37	2.76	4.36
16	2.69	3.35	2.74	2.91
17	3.08	2.45	3.75	2.90
18	4.21	3.27	3.45	3.74
19	2.60	2.44	3.07	2.60
20	2.74	3.82	2.74	2.92
Testing	3.78	4.18	3.72	3.29
Media	3.42	3.56	3.39	3.53
S	1.20	1.47	1.88	1.54

TABLE III. Concentration of Lead (ppm of dry matter).

Point of Sampling (n=3)	season			
	summer	autumn	winter	spring
1	13.24	12.18	15.68	14.36
2	12.85	16.23	15.22	12.82
3	14.55	13.50	11.94	13.57
4	12.25	14.78	13.54	11.47
5	13.08	12.95	13.85	15.14
6	13.02	12.18	13.80	15.14
7	12.32	13.40	13.59	14.36
8	13.25	12.90	15.20	12.82
9	13.23	13.55	15.68	11.47
10	12.83	12.10	13.85	11.45
11	12.87	13.03	13.54	11.49
12	12.23	12.18	11.94	13.52
13	12.27	12.95	15.22	12.87
14	13.05	13.50	11.96	13.47
15	13.11	16.23	11.86	11.57
16	13.01	14.78	13.64	12.92
17	13.15	12.10	13.50	12.72
18	12.20	13.58	13.58	12.80
19	12.30	13.40	13.44	12.84
20	12.25	12.18	12.04	11.47
Testing	14.23	12.74	13.08	13.84
Media	13.19	13.92	14.04	13.27
S	5.32	6.02	6.58	5.98

The average values of nickel, zinc and copper are always statistically higher in at sampling points in San Luis downtown than in the area control.

Nickel shows the maximum values in autumn, 34.95 ppm, and they differ statistically from other seasons of the year (Table IV). The area of lichen extraction shows a great variability

in the concentrations of zinc along the year (Table V). However, in all the seasons, the sampling points located in San Luis city always contain higher concentration of zinc. In some places this concentration is extremely high. The particles of zinc came from industrial resources and from the abrasion of car tyres (González and Pignata 1994).

TABLE IV. Concentration of Nickel (ppm of dry matter).

Point of Sampling (n=3)	season			
	summer	autumn	winter	spring
1	26.00	14.70	30.65	20.78
2	11.66	34.43	12.75	6.93
3	19.40	46.74	25.90	12.59
4	6.54	20.27	21.55	10.50
5	5.89	37.29	14.72	6.52
6	26.34	24.93	17.82	9.98
7	23.75	35.05	19.35	11.57
8	34.36	26.61	21.15	11.73
9	24.28	33.47	25.19	13.71
10	25.69	28.35	3.84	2.45
11	9.42	62.18	3.47	4.42
12	36.14	35.08	19.15	6.91
13	27.70	16.65	29.96	11.32
14	31.49	21.49	13.31	11.58
15	32.74	43.59	25.75	16.45
16	4.87	46.47	15.02	12.50
17	28.12	34.84	19.24	11.37
18	34.11	63.38	25.31	17.98
19	22.50	29.17	19.54	11.75
20	22.78	44.26	22.52	17.57
Testing	5.05	20.81	4.31	4.43
Media	22.69	34.95	19.32	11.43
S	9.96	14.10	8.01	4.84

TABLE IV. Concentration of Zinc (ppm of dry matter).

Point of Sampling (n=3)	season			
	summer	autumn	winter	spring
1	21.31	22.56	36.66	14.40
2	174.30	76.47	149.20	182.76
3	21.36	22.57	14.80	15.36
4	24.14	16.10	17.08	73.58
5	42.94	37.77	42.39	16.57
6	51.77	16.81	25.77	21.45
7	25.78	35.84	32.14	73.78
8	28.75	18.74	11.29	16.03
9	41.24	17.35	18.20	19.06
10	35.27	15.30	14.04	20.31
11	165.74	59.24	24.21	153.03
12	27.33	34.55	31.87	12.12
13	34.05	21.80	24.15	73.78
14	54.23	18.44	23.25	73.44
15	229.61	14.47	15.39	195.93
16	47.81	21.04	21.69	17.25
17	21.86	35.74	31.87	73.54
18	179.64	198.15	41.15	209.54
19	68.28	18.93	32.04	15.38
20	68.10	14.03	31.80	195.43
Testing	23.28	7.37	17.88	19.40
Media	68.18	35.80	31.94	73.63
S	63.76	43.82	30.71	76.26

The concentration ranges for nickel detected in the control area, are similar to those obtained by Garty et al (1996) using *Usnea hirta* (L.) lichen, 7,6 ppm. The zinc values reported in this paper greatly differ from those obtained by Garty.

The copper concentrations detected (Table VI) in San Luis downtown are bigger than the ones obtained in the control area, during the autumn and winter seasons. Comparing our results with other studies, it can be seen that *Usnea densirosttra* (Tayl.) 2.89 ppm, shows a similar accumulation than *Usnea filipéndula*, 2.34 ppm, used by Miszalski and Niewiadomska (1993), but it differs from the specie *Usnea hirta* (L.), 6.1ppm, used by Garty (1996).

The concentration of heavy metals in lichens

exposed in different points of microcenter tends to vary during the year. This is, probably, due to physiologic changes produced by modifications in environments humidity in the four seasons. These modifications are rather drastic in semiarid zones.

The sample points 18 and 20 are the most exposed to atmospheric contamination due to the intense transit they must support. However, these points do not lower registry such high contamination concentration values. The reason is that the contamination of the urban structure of the city and the air currents (natural and artificial) produces micro metrological phenomena which can disperse or concentrate contamination depending on both factors.

TABLE VI. Concentration of Copper (ppm of dry matter).

Point of Sampling (n=3)	season	
	autumn	winter
1	3.30	22.85
2	7.04	7.87
3	3.55	5.46
4	4.14	3.36
5	5.71	5.52
6	3.98	6.53
7	4.68	6.68
8	5.99	6.77
9	4.05	5.69
10	4.07	2.74
11	6.10	3.90
12	4.75	6.69
13	4.70	6.25
14	3.67	6.38
15	4.86	3.81
16	6.06	12.67
17	4.75	6.82
18	3.64	4.05
19	4.74	6.82
20	4.37	4.48
Testing	2.95	2.69
Media	4.71	6.77
S	1.06	4.58

CONCLUSIONS

From the analysis and comparison of the results, it can be stated that the use of lichens as bioaccumulator of heavy metals associated to a last generation determinative methodology such as ICP-AES, allows the monitoring of such elements in the environment.

The lichen *Usnea densirostra* (Tayl) has demonstrated to be a good biosensor of heavy metals principally nickel, zinc and copper. It has a bioaccumulation capacity similar to another analogous species or even superior.

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